

CLAIMS

The invention claimed is:

1. A method of generating a cell composition containing cardiomyocytes or cardiomyocyte precursor cells from primate pluripotent stem (pPS) cells obtained from a human blastocyst, comprising:
 - a) initiating differentiation of the pPS cells in suspension culture by forming embryoid bodies;
 - b) culturing the initiated cells so that they differentiate into areas that undergo spontaneous contraction;
 - c) harvesting the differentiated cells;
 - d) separating the harvested cells into fractions according to their density; and
 - e) collecting the cell fractions cells that express cardiac troponin I (cTnI), cardiac troponin T (cTnT), or atrial natriuretic factor (ANF) from an endogenous gene; thereby generating a cell composition containing cardiomyocytes or cardiomyocyte precursor cells.
2. The method of claim 1, wherein the embryoid bodies are plated onto a surface coated with gelatin or Matrigel®.
3. The method of claim 1, wherein the cells are differentiated in the presence of a nucleotide analog that affects DNA methylation; such as 5-aza-deoxy-cytidine.
4. The method of claim 1, wherein the cells are differentiated in a growth environment comprising a morphogen such as activin, and two or more growth factors.
5. The method of claim 4, wherein the morphogen is an activin, and the growth factors include an insulin-like growth factor and a member of the TGF β family.
6. The method of claim 1, wherein the cells are differentiated in a growth environment containing about 20% serum or serum substitute.
7. The method of claim 1, wherein the harvested cells are separated by density centrifugation.
8. The method of claim 1, wherein the separating comprises distributing cells in the population according to their density, and collecting cells at a density between ~1.05 and ~1.075 g/mL.
9. The method of claim 1, further comprising culturing the collected cells for at least 1 week in a medium containing a compound capable of forming a high energy phosphate bond, an acyl group carrier molecule, and a cardiomyocyte calcium channel modulator.

10. The method of claim 9, further comprising culturing the collected cells for at least 1 week in a medium containing creatine, carnitine, or taurine.
11. The method of claim 1, wherein the cell composition containing cardiomyocytes or cardiomyocyte precursor cells is further processed as follows:
 - a) cells that are present as single cells are separated from cells that are present as clusters; and
 - b) the cells present as clusters are resuspended in nutrient medium; and
 - c) the resuspended cells are recultured in the nutrient medium.
12. The method of claim 11, wherein the single cells are separated from the clustered cells by allowing the clustered cells to settle from suspension, and cells remaining in suspension are removed.
13. The method of claim 11, wherein the nutrient medium in which the resuspended cells are cultured contains about 20% serum or serum substitute.
14. The method of claim 11, in which clustered cells are separated, resuspended, and recultured three or more times.
15. The method of claim 11, wherein at least 60% of the clusters undergo spontaneous contraction after the reculturing.
16. A method of generating a cell composition containing clusters of cardiomyocytes or cardiomyocyte precursor cells from primate pluripotent stem (pPS) cells obtained from a human blastocyst, comprising:
 - a) producing a population of cells from the pPS cells in which at least 20% of the cells express cTnI, cTnT, ANF, or α -cardiac myosin heavy chain (MHC) from an endogenous gene;
 - b) separating cells that are present in the population as single cells from cells that are present as clusters;
 - c) resuspending the cells present as clusters in nutrient medium;
 - d) reculturing the resuspended cells in the nutrient medium;
 - e) sequentially repeating b), c) and d) so that each step is done three or more times; and
 - f) collecting and washing the cells after steps b), c) and d) are sequentially repeated; thereby generating cell clusters in which at least 80% of the clusters undergo spontaneous contraction.